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Bioremediation of toxic metal ions using biomass of *Aspergillus fumigatus* from fermentative waste

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Dried, nonliving, granulated biomass of *Aspergillus fumigatus* from fermentation industry was used for the removal of Cd^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} from solutions. Sorption studies showed sequestration (70-90%) of Cd^{2+} from solutions (0.1-4 m*M*). However, with increase in concentration, Cd^{2+} sorption efficiency decreased due to saturation of the biosorbent. Cu^{2+} binds most efficiently (72%) to the biosorbent followed by Cd^{2+} (61%), Co^{2+} (49%) and Ni^{2+} (37%). Metal removal from solutions containing a mixture of metal ions (Cd^{2+}, Cu^{2+}, Co^{2+}, and Ni^{2+}), which reflects the features of the polluted wastewaters and industrial effluents, was also efficient (90%) at lower concentrations (0.1 m*M* each). At higher concentrations (5 m*M* to 25 m*M*), Cu^{2+} removal was predominant (>70%) over other ions. The biosorbent was reusable up to 5 cycles with a 50% loss of initial Cd^{2+} binding capacity. However, a significant loss of Cd^{2+} binding capacity was observed when biosorbent was immobilized in polyvinyl foam. Infrared spectra of the biosorbent preparation showed the involvement of alcohol/amine (OH/NH_2) and CH-OH functional groups in metal binding. The present studies suggest that fungal biomass, a waste from fermentative industry, has the potential for removal/recovery of toxic metal ions from aqueous solutions.

Keywords: *Aspergillus fumigatus*, binding capacity, bioremediation, biosorption, metals

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Introduction

The threat of heavy metal pollution to public health and wild life has led to an increased interest in developing systems that can remove or neutralize its toxic effects in soil, sediments and wastewater. Heavy metals, even at low concentrations, can cause toxicity to humans and other forms of life[1]. Unlike organic contaminants, which can be degraded to harmless chemical species, heavy metals cannot be destroyed. The effects of metals on functioning of ecosystems vary considerably and have economic and public health significance. The main sources of toxic metal ions in streams are effluents from industries, such as electroplating, paints, plastics and battery. By most countries, cadmium, mercury and lead are included among the "priority pollutants" because of their high toxicity, requiring suitable treatment prior to discharge into the environment. Eventually, environmental awareness is growing among consumers and industrialists and legal constraints on discharge of effluents, necessitating a need for cost-effective alternate technologies.

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At present, a variety of physico-chemical processes are employed to treat toxic metal containing effluents. These processes, however, prove expensive when situations involving high volume and low metal concentration (typically less than 50 mg/l) are encountered[1]. Removal of toxic metal ions from polluted effluents by using microbial biomass is being studied extensively[2,3]. Both live and inactivated microbial biomass of bacteria, fungi and algae have been utilized for removing toxic metal ions[4-6]. The functional groups involved in the binding of heavy metals to microbial cells are phosphates, carboxyl, hydroxyl groups[7,8]. Complexation of metals by these ligands is by physico-chemical adsorption on the surface. Such biological metal removal process (biosorption) has distinct advantages over conventional methods. For example, the process does not produce chemical sludges (non-polluting), could be highly selective, more efficient, easy to operate and hence, cost effective for the treatment of large volumes of waste waters containing low concentrations[9].

Fermentation industries all over the world generate huge amounts of waste biomass, which are used in animal feed, organic manure or incinerated. The

potential use of waste biomass in metal recovery/removal remains largely untapped. However, such studies have recently enticed considerable interest among researchers. Mycelial wastes of *Rhizopus arrhizus*[10], *Penicillium chrysogenum*[11] and *Streptomyces pimprina*[12] have been studied extensively in the toxic metal removal processes. Such an alternative is economical and also attractive as disposal of fermentative waste itself remains a serious problem.

The aim of the present study is to evaluate the potential of *Aspergillus fumigatus*, a fermentative waste of antibiotic producing industry, for removing heavy and toxic metal ions from aqueous environment.

Materials and Methods

Strain/Biomass

The spent mycelial waste biomass of an antibiotic producing strain of *A. fumigatus* was a generous gift of Artemis Pharmaceuticals Limited, HAD, Jeedimetla, Hyderabad. The biomass was washed extensively with water, sun dried and used for preparation of biosorbent.

Preparation of Biosorbent and Metal Removal Studies

Sun dried flakes of fungal biomass were treated with boiling KOH (5%) for 15 min. The biosorbent was washed extensively with distilled water to neutral *p*H and stored at 4°C until further used. To determine the metal binding capacity, *A. fumigatus*-biosorbent (200 mg dry wt) was suspended both in 10 ml Cd^{2+} solutions (0.1-40 m*M*) and in 25 ml solutions (0.1-25 m*M*) of a mixture of metal ions (Cd^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+}). They were then incubated in a rotary shaker incubator (100 rpm) for 1 hr[2,3]. The biosorbent was washed with distilled water and bounded metal was eluted with 0.1 *M* HCl (20 ml) and estimated by Atomic Absorption Spectrophotometer (AAS, Perkin-Elmer 2380). In metal ion removal experiments the metal remaining in solution after incubation was estimated by AAS and percent removal was calculated.

Regeneration of Biosorbent

The biosorbent (1 g wet wt) was allowed to adsorb metal ions separately from 25 ml solution (25 m*M*) for 1 hr. The bound metals were desorbed with 0.1 *M* HCl (10 ml), washed extensively with distilled water and resuspended in similar molar solutions of metal ions for 1 hr. Metals were again desorbed with 0.1 *M* HCl and metal binding ability was reexamined. This procedure was repeated for 5 cycles.

Immobilization of Biosorbent

To 25 ml of polyvinyl alcohol solution (PVA, 10%), the following reagents were added: 1.5 ml glutaraldehyde (25%), 2 ml of methanol (50%), 3 ml of glacial acetic acid (10%), 1 ml of H_2SO_4 (1%), and thoroughly mixed using a magnetic stirrer. Alkali-extracted fungal biomass (1g wet wt) was added to the above mixture, suspended uniformly and poured drop-wise in methanol solution to obtain beads[8]. The beads were washed extensively with distilled water and stored at 4°C until further used.

Fourier Transform Infra Red Spectroscopy (FTIR)

Biosorbent preparations (control and metal-bound) were dried under nitrogen and ground with potassium bromide to obtain pellets which are subjected to Fourier Transform Infra Red (FTIR) Spectroscopy using Perkin-Elmer FTIR (Perkin-Elmer model No. 337) Spectrophotometer.

Unless indicated, the data shown are average values from three separate experiments. Variations up to 10% were observed.

Results and Discussion

Metal binding capacity of *A. fumigatus*-biosorbent, prepared from alkali extraction of the fermentative waste biomass, was investigated in solutions containing 0.1-40 m*M* Cd^{2+}. The results indicate that binding of Cd^{2+} to biosorbent increased with increase in concentration of solutions up to 30 m*M*, thereafter it got saturated (Fig. 1). At near saturation (30 m*M*), Cd^{2+} binding accounted for nearly 7% on a dry wt

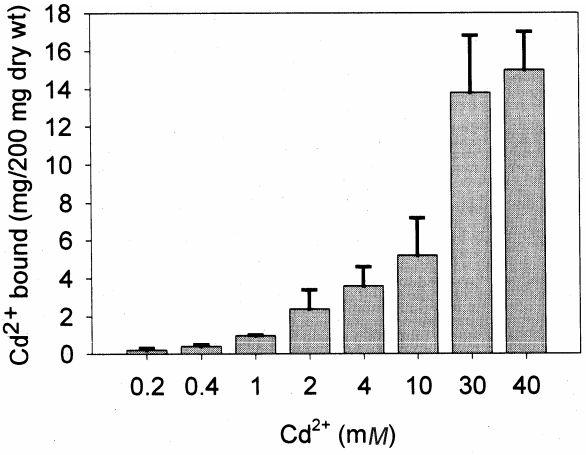

Fig. 1—Cd^{2+} sorption by *A. fumigatus*-biosorbent

basis. An interesting feature of the *A. fumigatus*-biosorbent was that most of the metal (70-90%) from Cd^{2+} solutions (0.1-40 m*M*) bounded to the biosorbent in 1 hr incubation period. Furthermore, Cd^{2+} was efficiently removed (99%) from very low concentrations (0.2 m*M*); however, with the increase in concentration, the metal removal efficiency was gradually decreased. At 30 m*M* concentration, the Cd^{2+} removal was calculated to be only about 40%, suggesting that metal-binding sites of the biosorbent has got saturated. Equilibrium sorption isotherm studies, using fermentative waste of *Streptoverticillium cinnamoneum*, showed that metal uptake has been a chemically equilibrated and saturable mechanism[13]. Thus, sorption increased with increase in concentration as long as binding sites were available. In the present study, a similar observation was also made with respect to Cd^{2+} binding.

A. fumigatus biosorbent was suspended separately in 25 ml metal ion solutions (25 m*M*) to test metal binding capacity of various metal ions. The results show that copper binds most efficiently (72%) to the biosorbent, followed by Cd^{2+} (61%), Co^{2+} (49%) and Ni^{2+} (37%) (Fig. 2). Metal removal was further studied from solutions containing mixture of metal ions of Cd^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+}, which reflected the features of the polluted wastewaters and industrial effluents. More than 90% of metals were removed from the mixture containing lower concentrations of metal ions (0.1 m*M*) (Fig. 3). While at higher concentrations (>5 m*M*), competitive effect was noticed between metal ions; Cu^{2+} was more efficiently removed (70%) than other ions. In a study with immobilized *Pseudomonas putida*, it has been reported that Cu^{2+} biosorption required only a few minutes to reach 90% efficiency[14].

Adsorption mechanism for metal ions deals with the transfer of metal from solution to the biosorbent surface[15]. The first step, bulk transport of metal ions in the solution phase, is usually rapid because of mixing and advective flow[16]. The second step, film transport, involves diffusion of the metal through a hypothetical film of hydrodynamic boundary layer around the biosorbent surface. The third step, actual adsorption of metal ions by the active sites of the biomass, is considered to be rapid and equivalent to an equilibrium reaction[17]. In the present study, a good mixing of solutes and biosorbent in the system helped to reduce the kinetic limitation due to the first step. However, kinetics of metal binding seems to be more

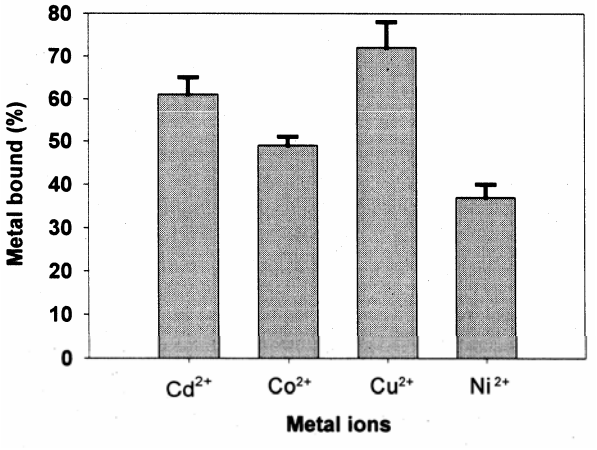

Fig. 2—Biosorption of metal ions

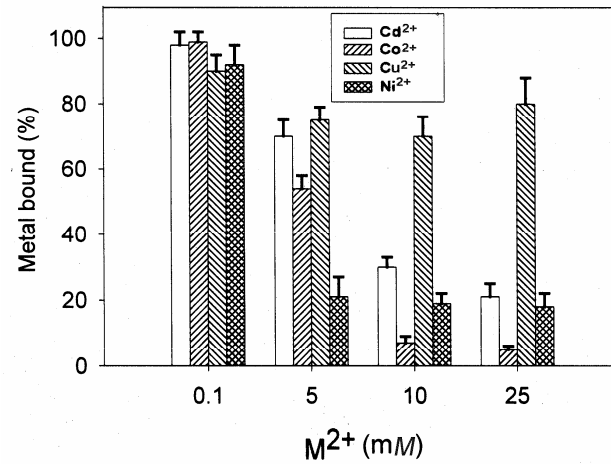

Fig. 3—Metal ion biosorption from amixture

seriously influenced by the transfer of metal ion from solution to active sites on the biosorbent surface.

The functional groups involved in metal ion binding are located in complex environment and it is a well-known fact that these sites have higher affinity towards transition metal ions as compared to alkali earth metal ions (Na, K and Ca). Hence, biosorbents are able to bind preferentially the toxic metal ions (Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} etc) while ion-exchange resins cannot distinguish between Na^{+}, K^{+} or Ca^{2+} and toxic metal ion[18]. The high affinity towards Cu^{2+} in the present study could be due to relatively high affinity to the binding groups. However, further studies are required to resolve this complex feature. In case of *Nitella flexilis*, two different sites exist on the cell walls, one with a high affinity and the other with a low affinity for Cu^{2+} binding. The high affinity sites are being more covalent in nature than low affinity sites[19]. In a recent study, pretreatment of

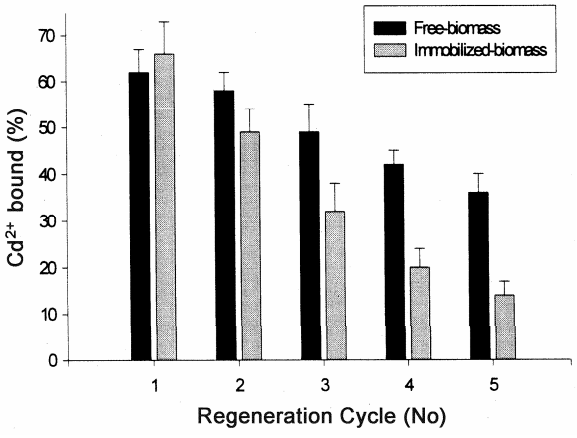

Fig. 4—Metal removal by regenerated *A. fumigatus*-biosorbent

Streptoverticillium cinnamoneum biomass, mycelial waste from a fermentation industry, with boiling water for 15 min increased the biosorption of lead and zinc by 52 and 41%, respectively[13].

The reusability of the fungal biosorbent was studied up to five cycles of sorption-desorption. The results indicate that up to 4 cycles of reuse, about 65-70% of the initial binding capacity of Cd^{2+} was retained; thereafter it decreased to 50% (Fig. 4). Immobilized biosorbent in polyvinyl alcohol polymer beads (at 1:1 ratio) was also tested for its ability to remove Cd^{2+} from solutions (25 m*M*). The results show that immobilized biomass was as efficient as biosorbent, binding up to 66% of Cd^{2+} and 58% of Co^{2+} (data not shown). However, when the same was reused several times there was a drastic loss of binding capacity by the 5^{th} cycle, which accounts to about 80% loss of initial binding (Fig. 4).

Immobilized biosorbents have been suggested to have obvious advantages of improved strength and handling capacity with reduced likelihood of system blockage, elevated pressure drops and better regeneration characteristics. However, the decrease in the loading capacities following desorption could be due to a variety of factors, such as loss of biomass from the reactor, structural damage to the biomass and blockage of binding sites by the metal complexes. It has been reported that treatment of the biomass with acids caused structural damage to the biomass[20]. If the biomass loss could be prevented it would help improve the process efficiency and also make it more economically attractive. For better shelf-life, the immobilized biomass has the advantage of easy and convenient usage compared to free biomass, which is easily biodegradable.

Infra red spectroscopic analysis of the biosorbent showed a sharp peak at 3385.7 cm^{-1} in the metal-free system. However, it transformed into a broad peak from 3216 to 3567 cm^{-1} in metal-bound systems, implicating NH_2 or OH groups in binding the metal with the biosorbent. Also the alcoholic C-O frequencies present at 1034.8 cm^{-1} in the metal-free biosorbent were transformed into broad peak between 1000 and 1150 cm^{-1} in the metal-bound biosorbent, indicating involvement of CH-OH groups. Almost similar results were obtained in case of *A. niger*[2] and *Phormidium valderianum*[8], an algal biosorbent. Biphasic plots were obtained for copper, cadmium and cobalt binding to *Saccharomyces cerevisiae* surface. However, it was difficult to assign maximum number of binding sites as each subset of sites had its own affinity constants[21]. Further, two non-equivalent binding sites were reported in the copper adsorption on the surface of *Rhizopus arrhizus*[22]. Recent studies from our laboratory with esterified *A. niger*-biosorbent indicated loss of binding of mercury and methyl mercury (>80%), which was regained when the ester groups were removed by alkaline hydrolysis, suggesting the involvement of carboxyl groups in the binding[23]. However, further studies are required to pinpoint the actual groups involved and their extent in metal binding.

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